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(21) International Application Number: PCT/US96/00459 (22) International Filing Date: 16 January 1996 (16.01.96) (30) Priority Data: 376,318 20 January 1995 (20.01.95) US (60) Parent Application or Grant (63) Related by Continuation US 376,318 (CIP) Filed on 20 January 1995 (20.01.95) (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CVETOVICH, Raymond [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). DEMCHAK, Richard [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). McCAULEY, James, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). VARSOLONA, Richard, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: THERMODYNAMICALLY STABLE CRYSTAL FORM OF 4"-DEOXY-4"-EPI-METHYLAMINO AVERMECTIN B1a/B1b BENZOIC ACID SALT AND PROCESSES FOR ITS PREPARATION**(57) Abstract**

The most thermodynamically stable crystalline form of the benzoic acid salt of 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b as the hemihydrate is obtained by crystallization from organic solvents containing a controlled amount of water.

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TITLE OF THE INVENTION

THERMODYNAMICALLY STABLE CRYSTAL FORM OF 4"-
DEOXY-4"-*EPI*-METHYLAMINO AVERMECTIN B1a/B1b
BENZOIC ACID SALT AND PROCESSES FOR ITS PREPARATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of our copending application Serial Number 08/109,189 filed 19 August 1993.

BACKGROUND OF THE INVENTION

The benzoic acid salt of 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b is known and known to be a stable salt as described in European Patent Publication EP 0465121-A1. The added stability relative to the stability of the previously described hydrochloride salt provided much greater shelf life for this important agricultural insecticide.

SUMMARY OF THE INVENTION

This invention is concerned with the most stable of four crystalline forms of the benzoic acid salt of 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b a known agricultural insecticide, and processes for preparing it.

The four crystalline forms that have been identified are designated as forms A, B, C and D, three of which are hydrated (B, C and D). The most thermodynamically stable crystalline form is the hemi-hydrate B. Knowing the most stable crystalline form and devising processes for producing the product in that form is extremely important in that it provides bulk material with crystal homogeneity that is not going to transform to another crystal habit on storage.

The novel crystal form B has been found to be significantly less hygroscopic than the other crystal forms and in addition it retains

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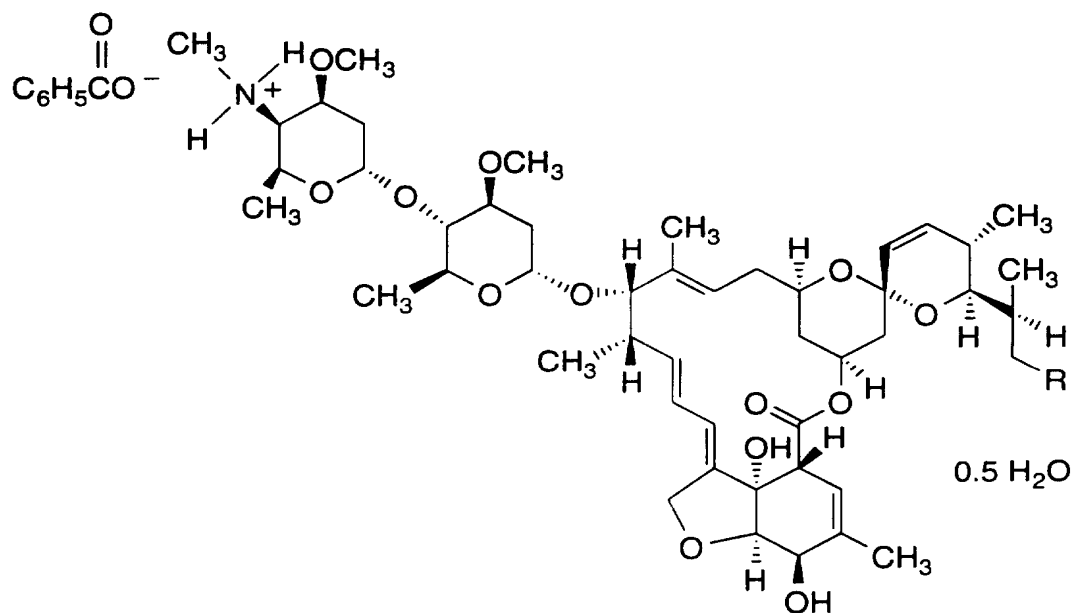
much less solvent. These attributes provide for a much more stable and uniform form of the final product which is used to prepare agricultural products and pharmaceutical dosage forms.

The novel processes for producing crystal form B comprises crystallization of the compound from an organic solvent containing a controlled amount of water.

DETAILED DESCRIPTION OF THE INVENTION

The novel compound of this invention is crystalline 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b, benzoic acid salt, hemihydrate in substantially pure form.

It has structural formula:



wherein R is -CH₃ (B1a) or -H (B1b) and is referred to as Crystal form B, Type B or similar designation.

The differential scanning calorimetry (DSC) curve determined at a heating rate of 20°C/min. under nitrogen flow in an open cup is characterized by a relatively broad water loss endotherm with a peak temperature of 74°C and a major melting/decomposition endotherm

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with peak temperature of 155°C, extrapolated onset temperature of 150°C with an associated heat of 56 Joules/gm. The x-ray powder diffraction pattern for Type B is characterized by d-spacings of 18.13, 9.08, 8.68, 5.03, 4.61, 4.53, 3.97 and 3.82Å. The aqueous solubility is pH dependent. At pH 5, acetate buffer, it has a solubility of 0.32 mg/ml.

The novel process of this invention comprises recrystallizing the compound in any energetic state from an aqueous organic solvent. Preferably form B is prepared from Form A.

The aqueous organic solvent useful in the novel process is preferably acetonitrile with 2-4% w/w of water; methyl t-butyl ether (MTBE) with 0.5 to 0.8% w/w of water; or isopropanol (IPA) with 0.1-0.3% w/w of water.

Compound of crystal form A, C or D is dissolved in the organic solvent at about 50-60°C treated with the requisite amount of water, seeded with Type B crystals, cooled to and aged at about 15-30°C for about 2-4 hours and further cooled to about 5°C over a period of about 1-2 hours.

The crystalline product is collected on a filter and dried *in vacuo*.

The utility and methods of use of the benzoic acid salt described herein and the free base thereof are well known by those skilled in the art and fully described in the scientific and patent literature.

It has significant parasitocidal activity as an anthelmintic, ectoparasiticide, insecticide and acaricide, in human and animal health and in agriculture. As an agricultural pesticide it has activity against insect pests of stored grains such as *Tribolium sp.*, *Tenebrio sp.*, and of agricultural plants such as spider mites, (*Tetranychus sp.*), aphids, (*Acyrtiosiphon sp.*); against migratory orthopterans such as locusts and immature stages of insects living on plant tissue. The compound is useful as a nematocide for the control of soil nematodes and plant parasites such as *Meloidogyne sp.* which is of importance in agriculture. The compound is active against other plant pests such as the southern army worm and Mexican bean beetle larvae.

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The compounds are applied using known techniques as sprays, dusts, emulsions and the like, to the growing or stored crops to effect protection from such agricultural pests. For the treatment of growing crops, the compound is administered at a rate of about 5-50 gms per hectare. For the protection of stored crops it is normally administered by spraying with a solution containing from 0.1-10 ppm. of the compound.

The following examples are provided in order that the invention might be more fully understood. The examples are not to be construed as limitations upon the scope of the invention.

EXAMPLE 1

5-O-t-Butyldimethylsilyl avermectin B1a/B1b

A solution of 50 g of avermectin B1a/B1b (dried over P₂O₅ in high vacuum to constant weight), 24 g of imidazole and 24 g of tert-butyldimethylsilyl chloride in 400 ml of anhydrous N,N-dimethylformamide was stirred at room temperature for 50 minutes. The reaction mixture was poured into 1.5 l of ice cold water and the aqueous phase was extracted four times with 200 ml of ether. The organic phase was washed twice with water, aqueous sodium chloride solution, dried with magnesium sulfate and concentrated *in vacuo* to a white foam. The crude product was purified by silica gel column chromatography, with a methylene chloride:ethyl acetate, 90:10 to 70:30 solvent system to give 46.5 g of 5-O-t-butyldimethylsilyl avermectin B1a/B1b as an amorphous foam, which was characterized by its ¹H-NMR and mass spectra.

EXAMPLE 2

5-O-t-Butyldimethylsilyl-4"-oxo avermectin B1a/B1b

To a solution containing 9.1 ml of oxalyl chloride in 230 ml of dry methylene chloride stirred at -60°C, was added 15 ml of dry dimethylsulfoxide dissolved in 120 ml of dry methylene chloride during 15 min. Then a solution of 46.5 g of 4-O-t-butyldimethylsilyl avermectin

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B1a/B1b dissolved in 230 ml of dry methylene chloride was added over a period of 15 minutes while maintaining the temperature at -60°C. The reaction mixture was stirred at this temperature for 30 minutes when 65 ml of dry triethylamine was added. The mixture was stirred for 5 additional minutes at -60°C, the cooling bath was removed and the reaction mixture was allowed to come to ambient temperature. After addition of water the reaction product was extracted with methylene chloride, the extract was washed with water, dried and concentrated *in vacuo* to 45.5 g of a yellow foam. This was identified by its mass and NMR spectra as 5-O-t-butyldimethylsilyl-4"-oxo avermectin B1a/B1b, which was used for further chemical reactions without purification.

EXAMPLE 3

4"-Deoxy-4"-*epi*-methylamino-5-O-t-butyldimethylsilyl avermectin B1a/B1b and
4"-deoxy-4"-methylamino-5-O-t-butyldimethylsilyl avermectin B1a/B1b

A solution of 26 ml of glacial acetic acid in 300 ml of methanol was treated with methylamine gas at 0°C, until the pH of the solution reached 9.0. To this a solution containing 44.5 g of 5-O-t-butyldimethylsilyl-4"-oxo avermectin B1a/B1b in 200 ml of methanol was added, and the reaction mixture was stirred at room temperature for 1 hour, when a solution of 3.5 g of sodium cyanoborohydride in 75 ml of methanol was added dropwise over 10 minutes. After 50 minutes the reaction mixture was poured into 1.5 l of cold aqueous sodium carbonate solution and the product was extracted with ether. The extract was washed with water, dried, and concentrated *in vacuo* to 44.8 g of yellow foam. Thin layer chromatography (silica gel, ethyl acetate: methylene chloride, 85:15) of the crude product at this point shows several spots. Further purification by silica gel column chromatography using ethyl acetate solvent mixtures gave 4.7 g of 5-O-t-butyldimethylsilyl-4"-*epi* avermectin B1a/B1b, 1.2 g of 4"-deoxy-4"-methylamino-5-O-t-butyldimethylsilyl avermectin B1a/B1b, and 14 g of 4"-deoxy-4"-*epi*-

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methylamino-5-O-t-butyldimethylsilyl avermectin B1a/B1b as light foams, which were characterized by their mass spectrum and their ^1H -NMR, and ^{13}C -NMR spectra.

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EXAMPLE 4

4"-Deoxy-4"-*epi*-methylamino avermectin B1a/B1b

A solution of 14 g of 4"-deoxy-4"-*epi*-methylamino-5-O-t-butyldimethylsilyl avermectin B1a/B1b in 200 ml of methanol and a solution of 7 g of p-toluenesulfonic acid monohydrate in 500 ml of methanol was mixed and stirred at room temperature for 45 minutes, and then poured into dilute aqueous sodium carbonate solution. The product was extracted with ethyl acetate, washed with water and dried over magnesium sulfate, concentrated *in vacuo*, and purified by preparative silica gel column chromatography with a methylene chloride/methanol 95:5 solvent mixture to give 6.7 g of 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b, which was identified by NMR and mass spectra.

EXAMPLE 5

4"-Deoxy-4"-*epi*-methylamino avermectin B1b/B1a Benzoic Acid Salt (Type A material)

4"-Deoxy-4"-*epi*-methylamino avermectin B1a/B1b (5.10 kg, 5.75 m) in methyl tert-butyl ether (18 L) was treated with benzoic acid (7.55 g, 6.18 m) at 25°C. To this solution was added hexanes (36 L) over a 0.5-1.0 hour period, whereupon crystallization occurs during the addition. The crystalline slurry was cooled to 0°-2°C, aged for 1 hour at 0°-2°C, then filtered. The filter cake was then washed with a mixture of methyl tert-butyl ether/hexanes (1:2) and dried *in vacuo* at 60°C. to give 5.7 kg of 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b benzoic acid salt (Type A).

Molecular Weight: 1008

Melting Point: 133°-136°C.

Microanalysis: calculated: C, 66.71; H, 8.10; N, 1.39

found: C, 66.93; H, 8.32; N, 1.20.

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EXAMPLE 6

Preparation of Type B Product

<u>Materials</u>	<u>Amounts</u>
	3.59 Kg 94.2 wt%
TYPE A MATERIAL (obtained by crystallization from MTBE as described in Example 5)	2.33 Kg 95.7 wt%
	5.92 Kg (5.61 Kg by assay)
ACETONITRILE (HPLC GRADE)	40 L (KF = 0.2 mg/ml)
WATER (DI)	570 mL

Procedure

A 50 L 4-neck round bottom flask was equipped with a mechanical stirrer, N₂ inlet, thermocouple and funnel. After purging the vessel with N₂, acetonitrile (12 L, KF = 0.2 mg/ml) was added and warmed to 50-55°C, and Type A material was charged. Upon complete addition (30 minute), the mixture was aged an additional 10 minutes in a nitrogen atmosphere to give a clear red-brown solution.

The batch was diluted with additional acetonitrile (6 L), water (270 mL) was added, and then seed crystals of form Type B (10 g) were added at 45°C. Crystal growth began immediately and rapidly at 45°C. Crystallization must initiate, and largely occur at >35°C to assure formation of Type B crystals.

The batch was cooled to 20°C over a 45 minutes period and aged for 2.5 hrs. The crystalline slurry thickened during this period and made stirring difficult. Additional acetonitrile (2 L) was added, and the slurry was cooled to 5°C over 1 hr. After aging at 5°C for 30 minute the slurry was filtered onto a 22-in. diameter Cenco-Lapp funnel lined with paper and polypropylene. The mother liquor was recycled back to the crystallization vessel to rinse and transfer the remaining product. The

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cake was washed with cold (2°C) wet acetonitrile (5 X 4 L, each 1.5% (v/v) in water). The cake was partially dried with a stream of nitrogen passed through the cake for 30 minute and it was then transferred to the Hull dryer.

The cake was dried at 30°C *in vacuo* (25 in.) over 3 days to a constant weight and an acetonitrile content <0.1 wt% by GC. The batch product was 4.95 Kg of Type B material.

Analyses B1a anhydrous Type B

MW	1008.26	
FORMULA	C ₅₆ H ₈₁ NO ₁₅	
CFA:	OFF-WHITE POWDER	
X-RAY	CRYST. TYPE B HEMIHYDRATE	
HPLC*	97.1 WT%	
	97.8 area%	
TLC	SINGLE SPOT	
TITRATION*	100% (Perchloric acid)	
UV*	A% 333, 245 nm in methanol	
KF	1.08 wt%	
GC	CH ₃ CN	<0.01%
	HEXANES	<0.01%
	MTBE	nd, <0.01%
	IPAc	nd, <0.01%

*corrected for KF, GC

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WHAT IS CLAIMED IS:

1. A crystalline compound 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b, benzoic acid salt, hemihydrate Type B in substantially pure form.
2. The crystalline compound of Claim 1 which is 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b, benzoic acid salt, hemihydrate in substantially pure form characterized in that differential scanning calorimetry at a heating rate of 20°C per minute characterizes a water loss endotherm with a peak temperature of 74°C and a melting/decomposition endotherm with a peak temperature of 155°C and an extrapolated onset temperature of 150°C and an associated heat of 56 Joules per gram; an X-ray powder diffraction characterized by d-spacings of 18.13, 9.08, 8.58, 5.03, 4.61, 4.53, 3.97 and 3.82Å; and in pH5 acetate buffer a solubility of 0.32 mg per ml.
3. The process for the preparation of the crystalline compound 4"-deoxy-4"-*epi*-methylamino avermectin B1a/B1b, benzoic acid salt, hemihydrate (Type B) in substantially pure form which comprises recrystallizing Type A material from an aqueous organic solvent selected from acetonitrile methyl tert-butyl ether or isopropanol by dissolving the starting material in the organic solvent at a temperature of 50-60°C adding about 0.1 to 4% w/w of water; cooling to about 45-35°C; seeding with Type B crystals; holding until crystallization is essentially complete; cooling to and holding at 15 to 30°C for about 2-4 hours, cooling to about 5°C over a period of 1-2 hours, collecting the solids and drying.
4. The process of Claim 2 wherein the aqueous organic solvent is acetonitrile with 2-4% w/w of water; methyl t-butyl ether with 0.5 to 0.8% w/w of water; or isopropanol with 0.1 to 0.3% w/w of water.

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5. The process of Claim 2 wherein the aqueous solvent is acetonitrile with 2-4% w/w of water.

6. A method for the control of agricultural insects, which comprises applying to an area infested with such agricultural insects an effective amount of the stable salt of Claim 1.

7. A composition useful for the treatment of insect infestations of plants or plant products which comprises an inert carrier and an effective amount of the stable salt of Claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/00459A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H17/08 A01N43/90

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP,A,0 465 121 (MERCK AND CO., INC.) 8 January 1992 cited in the application see example 10 ---	1,3,6,7
A	JOURNAL OF ORGANIC CHEMISTRY, vol. 59, no. 25, 16 December 1994, EASTON US, pages 7704-7708, XP002003103 R.J.CVETOVICH ET AL.: "Syntheses of 4"-epi-amino-42-deoxyavermectins B1." see page 7707, column 2, line 69 - page 7708, column 2, line 17 --- -/--	1,3,6,7

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

15 May 1996

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NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Scott, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	JOURNAL OF ORGANIC CHEMISTRY, vol. 59, no. 25, 16 December 1994, EASTON US, pages 7870-7875, XP002003104 M.F.LOEWE ET AL.: "Glycosidation Route to 4"-epi-(Methylamino)-4"-Deoxyavermectin B1 (MK-244, Enamectin Benzoate)." -----	1,3,6,7
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